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EXPERIMENTS WITH A MAGNESIUM SEAWATER CELL INCORPORATING A BACTERIAL COLONIZED CATHODE

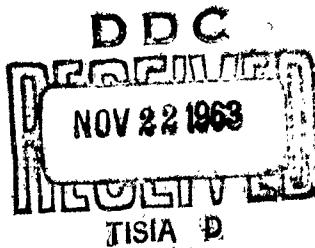
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ABSTRACT

A magnesium seawater cell, with its cathode covered by a colony of special bacteria, yields a higher voltage at a given current density than when bacteria are not present. This has been observed for current densities below one ampere per square foot. Volt-ampere characteristics were measured to determine how much the voltage performance improved, and if possible, why. On the basis of these tests, it is possible to conclude that the bacteria act to lessen cathodic polarization, and therefore the equivalent internal resistance of the cell, to an extent dependent on the hydrogen uptake coincident to their normal metabolism. A five-month life test in a clean, shallow oceanic environment revealed that the cell shut down to zero output, due apparently to calcareous deposits formed from two distinct actions: the cell's normal electrochemical process and superposed action by film-forming organisms.

PROBLEM STATUS

This is a final report on one phase of the problem on new methods of energy conversion; work in other areas is continuing.

AUTHORIZATION

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EXPERIMENTS WITH A MAGNESIUM SEAWATER CELL INCORPORATING A BACTERIAL COLONIZED CATHODE

INTRODUCTION

Since early 1960, there has been limited but lively interest in a novel type of electrochemical cell featuring live bacteria on the cathode. This interest was prompted by the Navy's search for a long-life, low-power, economical electric power source possessing maximum compatibility to an oceanic environment. The bacterial cell, or biocell, incorporating biological species whose effects were superimposed on the action of an ordinary seawater type of cell, was merely one of numerous systems considered. It was to be considered competitively against primary, secondary, and fuel cells, wave-action or pressure-actuated electromechanical devices, and direct conversion systems operated from special chemical or nuclear heat sources.

Certain attractive features of this so-called biocell are noted:

1. Such a seawater battery could be the ultimate in simplicity, which in turn is strongly correlated to reliability. Essentially it would comprise two plates open to the sea with seawater serving as the electrolyte. Shelf life prior to use could be indefinite.
2. Magnesium as the anode would provide a convenient, safe, and compact energy source, although perhaps not the most economical one compared to the fuels of competitive systems.
3. Mild steel as the inert cathode in this instance of the magnesium-hydrogen reaction, would be rugged, relatively inexpensive, and provide an excellent substrate for the bacteria, as it so often does around piers and sunken ship hulls. When coupled to the magnesium through the electrical load, it would not rust.
4. The locating of the bacteria on the steel cathode could significantly improve the magnesium seawater cell performance.
5. The bacteria are indigenous to the sea and would naturally continue to subsist on the nutrients present in the seawater electrolyte.

Obviously these features enticed investigators to look more closely into the feasibility of the biocell as a practical power source to meet the criteria of long life and economy.

Other factors posed restrictions or tempered enthusiasms:

1. The improved performance cited above was limited to low current densities, i.e., below 1 amp/ft².
2. The gain achieved by employing bacteria could probably be matched by treating the cathode with nickel, palladium, or platinum. However, later discussion will show that the bacteria improve the operation of cathodes plated with nickel. For operation in seawater they would probably also improve operation with palladium or platinum treated electrodes.

3. Because of the low current density limitation of the cell, its longevity capability should measure at least in months if it is to have any practicability.

With the study under way, a lively controversy developed centering around two principal viewpoints: (a) the bacteria were merely acting as depolarizers and thusly reducing the hydrogen overvoltage at the cathode; (b) bacteria were actually producing electricity, i.e., contributing an incremental equilibrium potential, or assisting the transport of charge across the electrode/electrolyte interface. An even more liberal viewpoint allowed the existence of both actions. Subsequent work has tended rather strongly to reinforce the first notion and disestablish the second. This is a rather painful conclusion to devotees of the second viewpoint, a viewpoint which implies that the ocean is potentially a vast source of fuel which may be converted to electricity by bacterial metabolism.

The ensuing discussion will treat the behavior of the biocell from an experimental standpoint, emphasizing the role played by the bacteria with respect to the overall operation of the one type of seawater cell considered, namely that employing the magnesium-hydrogen couple. Comparisons between identical cells with and without bacteria will be made. In these comparisons, the cell without bacteria will be considered as a reference, or control, on the basis that its reactions and performance is known. In order to assure that the control is identical in all respects except the bacteriological factor, tests will be presented with a cell whose bacterial culture has been killed but whose other characteristics, such as the matrix or matte formed on the cathode by the culture, remained intact. It will be shown that such an ultimate reference is nearly identical to more convenient control cells on which cultures have not been grown.

A great number of electrode materials and bacterial types were considered in the subsequent course of the work, but because of its superior performance, the magnesium seawater cell with sulfate-reducing bacteria colonizing the cathode proved to be the most suitable for further study to determine the mechanisms of action taking place and the overall performance capabilities.

The experiments reported here were performed with this type of biocell. From them an estimate has been made of the performance capabilities. Roughly a doubling of power is possible under favorable oceanic conditions. Of course the total energy is fixed by the quantity of anode magnesium. Tests were also made toward confirming the fact that the performance enhancement was due to the action of live bacteria and not solely to chemical by-products formed in the cell during the course of its operation in the presence of the bacteria.

The action of the magnesium seawater cell will also be reviewed for convenience and will illustrate the reactions and the electron and ion transport. Finally, the results of life tests on the biocell and control cell in an ocean environment will be presented along with an analysis of the deposits formed on the cell electrodes, which appear to be responsible for performance degradation over a several-month period.

The aspects of cell longevity have not been resolved, but some limited tests and subsequent analysis of results indicate that deposits of a calcareous nature build up on the cells, at least those which were tested, and degrade performance over a several-month period.

BACKGROUND

Electrochemical action involving bacteria dates back to 1911 with the study by Potter (1) and work in the thirties by Cohen (2) and Von Wolzogen Kuhr and van der Vlugt (3). This last work accounted for the bacterial action in a manner consistent with the microbiological studies of Sisler and Zobell (4) reported in 1950. Further work during 1960-1962

by Booth, Tiller, and Wormwell (5-7), oriented toward corrosion phenomena, treated the action of the bacteria in terms of their influence on half-cell potentials.

The origin of this recent incorporation of bacteria into a common seawater cell is uncertain. Both Rohrbach* and Sarbacher† were apparently active along similar lines in the 1959-60 period. Their approach consisted of growing a mixed culture of sulfate-reducing bacteria on an iron electrode and coupling this electrode to an adjacent magnesium anode through an external load.

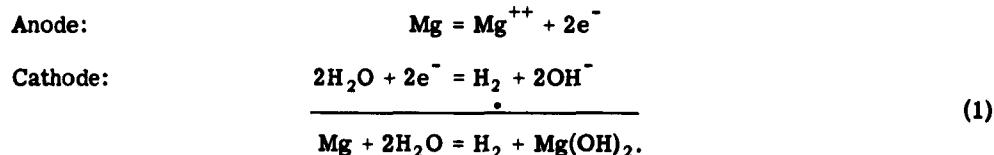
The basic knowledge to account qualitatively for the biocell's action was known prior to the 1959-60 period. The polarization characteristic of magnesium in a brine electrolyte approximating seawater was known, although not too widely, from the work by Armour (8). The iron or mild steel cathode behavior was known from an abundance of earlier corrosion studies (9). The role of the bacteria could be inferred from the work of Von Wolzogen Kuhr and van der Vlugt (3) and Sisler (4).

These facts notwithstanding, few investigators rushed into publication to explain the overall action of the biocell as an electricity producing device. This can best be appreciated only if one experienced the tension and uncertainty on the issue of whether the bacteria were directly producing electrical energy or indirectly doing it by creating certain chemical products in the course of their metabolism. The press was on the scene, to be sure, with several articles highlighting the drama of bugs generating electricity and the revolutionary consequences thereof. In retrospect, these now seem somewhat ill-advised and certainly premature.

The meticulous experimental studies by Booth and Tiller (5,6) in 1960-62, oriented toward problems in the field of corrosion of steel, provided some powerful quantitative data in support of the depolarizing theory and in opposition to the electricity-producing theory. The work reported here is a further step in that direction and, although not conclusive, resolves additional points contributory to an overall mechanistic model of the biocell.

THE MAGNESIUM SEAWATER CELL

The reactions for a magnesium seawater cell with an inert cathode and basic electrolyte are generally accepted to be

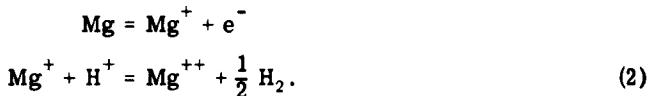


The principal value in defining these reactions rests in the noting of the quantities involved. For example, the anode reaction as written above is an idealized one connoting reversibility and a standard potential of 2.37 volts. Actually in seawater, a potential of about 1.5 volts (with respect to a saturated calomel electrode) is observed. Furthermore, hydrogen

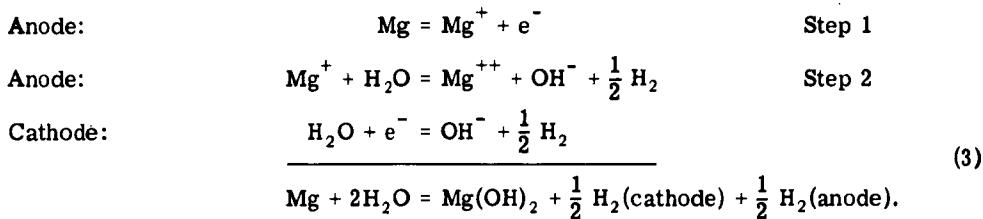
* Then Manager of the Research and Development Division, Magna Corporation.

† Early experiments around 1959 have been related orally by Dr. R. Sarbacher, President, General Scientific Corporation.

is evolved at the anode in quantities equal to that at the cathode for the low current densities considered in this discussion (8). The following relations have been formulated to portray more adequately what takes place:



The net ion balance is more clear if the relations are written



The consequences of these reactions are shown schematically in Fig. 1. The principal charge carriers in the electrolyte, Na^+ and Cl^- , are also shown, particularly in reference to the essential current continuity which must be accounted for. The reactions involved in both the electrode reaction and charge transport show that the pH level will remain unaffected, thus confirming the findings in the Armour study (8). Local pH changes occur in the region of the electrodes if electrolyte circulation is not adequate.

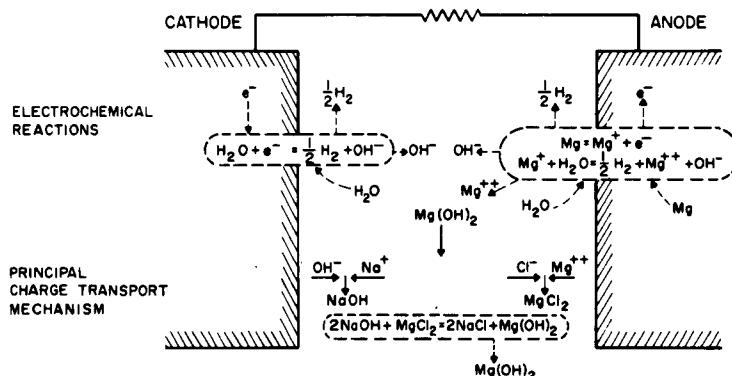


Fig. 1 - Schematic diagram of the magnesium seawater cell depicting the electrode reactions and the principal charge carrying mechanism in the electrolyte

OPERATION OF THE MAGNESIUM SEAWATER CELL WITH A BACTERIAL COLONIZED CATHODE

Comparative Performance of the Biocell and the Control Cell

Performance of the biocell is best appreciated by comparison with the plain magnesium seawater cell employed as a reference, or control. The biocell demonstrates a superior

performance over its respective control in all tests reported here. However, the absolute performance of the controls vary, as do the biocells, because of their basic construction differences.

Figure 2 shows comparative performances for two types of biocells. The cells both have similar magnesium anode materials but differing cathode materials, structural shapes, sizes, and bacterial cultures. Both have mixed cultures of sulfate-reducing bacteria consisting in the main of the genus *Desulfovibrio*. Set A applies to stable operation over approximately a one-week duration. The cathode was untreated mild steel except, of course, that the biocell cathode was coated with a bacterial colony. Set B applies to short-term, but nevertheless stable, operation. Customarily, such comparative information is presented as half-cell polarization characteristics. However, the voltage-current curve for the overall cell is more useful to power source considerations. In addition, the half-cell characteristic for magnesium in seawater is essentially constant at about 1.5 volts with respect to the Saturated Calomel Electrode (SCE), particularly over the current range considered. Therefore, it is clear that polarization is taking place principally at the cathode.

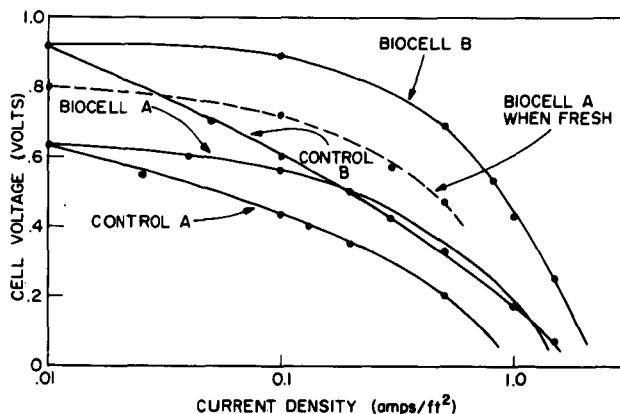


Fig. 2 - Comparative performance characteristics of biocells and corresponding controls

The performance gain with the presence of bacteria on the cathode is clearly evident from both sets of curves in Fig. 2. The magnitude of this gain is better illustrated by the power curves of Fig. 3. The ratio of maximum power densities using values at the maximum power points is 1.8 and 2.38 for the two sets of cells.

The noticeable difference in average performance level indicated in Fig. 2 for the two sets of biocells is due to different electrode spacing, cathode preparation, and duration of operation. The B cells possessed a nickel plating over the mild steel base. An even greater improvement could have been achieved with palladium or platinized platinum cathode surfaces. The A cell cathodes were plain mild steel. The A cells also degraded considerably over the week's period during which they operated because of electrode deposits, which later will be argued as carbonates. When the A cells were comparably new with respect to the B cells, except for some differences in the condition of the Mg anode, the biocell A performed as shown by the dotted curve in Fig. 2.

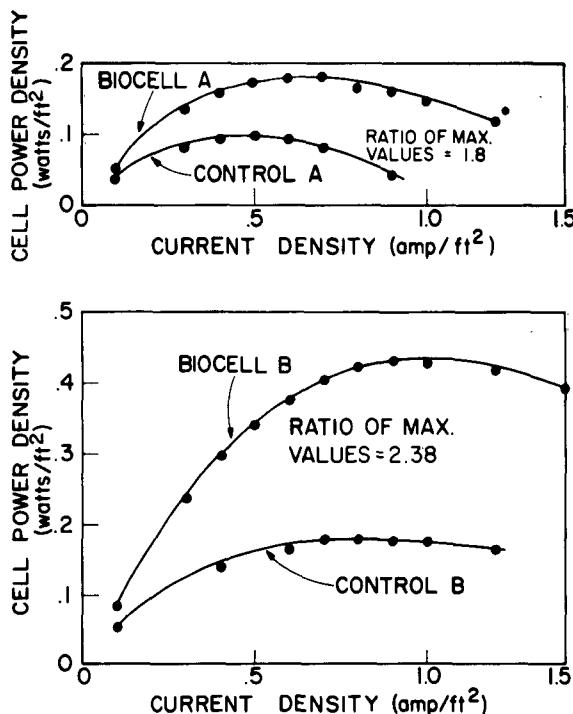


Fig. 3 - Comparative power densities derived from Fig. 2

Analysis of Performance Difference

Why is the performance of the biocell superior to its control counterpart? Based on the evidence presented in Fig. 2, it appears to be due to either a reduction in the hydrogen overvoltage for the cathodic reaction in this instance or an enhancement in the charge-carrier transport by the electrolyte at non-zero current values. The latter may be ruled out by virtue of the fact that the electrolyte properties are kept constant between the two cell types, i.e., with and without bacteria. There is no evidence that the bacteria inject charge carriers into the electrolyte, thereby increasing its conductivity; they are present in appreciative quantities only on the cathode surface, since the electrolyte is flowing through the cell at a rate whereby the electrolyte volume is replaced every two minutes.

Figure 2 shows that at a given current density the voltage is higher for the biocell than for its corresponding control. It is postulated then that the hydrogen overvoltage on the cathode is reduced when live bacteria are on it. Recalling that there is near zero polarization on the magnesium anode helps to understand this. That the bacteria must be living is established in later discussion dealing with the effects of temperature. The barrier accounting for hydrogen overvoltage is somehow reduced. Since it is known that hydrogenase-containing *Desulfovibrio* ingest hydrogen as well as sulfate, this lessening of internal resistance is consistent with what one would expect with *Desulfovibrio* present. Later discussion will bring out that the enzyme hydrogenase identifies the *Desulfovibrio* species, which are of the class of hydrogen-consuming sulfate-reducers.

Another phenomenon observed from these curves is that the reduction of internal resistance, or hydrogen overvoltage, is greatest below current densities roughly of 1 amp/ft²,

closer actually to 0.1 amp/ft². This advantage lessens as current density increases, probably because of the finite hydrogen uptake capability of *Desulfovibrio* and of other hydrogen-consuming bacteria which may be present as heterotrophs (10).

Actually this lower current range where bacteria are significantly effective impose an operational limitation: the *Desulfovibrio*-type magnesium biocell operates most advantageously over its nonbacterial counterpart in a seawater or simulated seawater environment at current densities less than 1 amp/ft².

The Question of Open Circuit Voltage

The effect of bacteria on cell open circuit voltage remains unanswered even though Fig. 2 appears to present equal no-load voltage values. From the curves there appears to be no difference in voltage at no load between the biocell and its control. However, these no-load values are actually values at currents approaching zero and are not to be construed as equilibrium voltages. They are the result of averaging the performance data from which the volt-ampere characteristics were derived.

There may be significance in the results presented, however. The net or average result of the tests show that the no-load voltage of the biocell does not differ from its control. This leads to the conclusion that the bacteria colonized on the cathode do not directly produce an incremental voltage at low current and therefore probably do not do so under open circuit conditions. Equilibrium potentials are difficult to obtain and securing good results calls for accuracy beyond that employed in these experiments. Nevertheless the statistical average represented in these curves reduce the scatter from the intrinsically inaccurate (approximately 5 percent) measurements from which the curves of Fig. 2 are derived; and although the no-load voltages do not represent equilibrium potentials, they indicate that no increment of potential may reasonably be attributed to bacteria.

A second conclusion to note from the tests with the magnesium seawater biocell is that the bacteria in the process of their metabolism apparently do not produce electricity which may furnish an increment of power to the biocell load.

Biological Function of Bacteria Relevant to Hydrogen Consumption

The studies by Booth and Tiller (5,6) on polarization of mild steel in cultures of sulfate-reducing bacteria strengthen the thesis discussed here. They establish that cathodic polarization is lessened because of bacterial action for sulfate-reducing species containing the enzyme hydrogenase, species similar to those utilized in these experiments. Whereas these studies were aimed at resolving questions in connection with the anaerobic corrosion of metals, they may be applied to considerations here involving the production of electricity by the magnesium seawater cell.

The studies of Booth and Tiller are summarized in Table 1. Although *D. desulfuricans* is a fresh water species, Sisler (4,10) has demonstrated that marine species such as *D. aestuarii* also possess the capability of consuming molecular hydrogen. The mixed culture used in the experiments reported here were derived from a marine environment.

There is a difference among the species listed above which de-emphasizes the role of sulfate reduction and strengthens that of hydrogen consumption. For *D. desulfuricans* the hydrogenase and sulfate reductase systems are interrelated, or coupled, but for *C. nigrificans* they are not. Yet in both instances where there is hydrogenase action, cathodic depolarization occurs (6). This suggests that bacteria other than sulfate reducers may function to depolarize the cathode as long as they are in possession of hydrogenase action.

Table 1
Characteristics of Sulfate-Reducing Bacteria

Genus and Species	Hydrogenase Containing	Sulfate Reduction	Cathodic Depolarization
Desulfovibrio Desulfuricans	Yes	Yes	Yes
Desulfovibrio Orientis	No	Yes	No
Clostridium Nigrificans			
Strain A	Yes	Yes	Yes
Strain B	No	Yes	No

Hydrogen evolution and consumption may be accounted for quantitatively. Although the results are estimates at best, they are derived in a logical manner and could be improved with better basic data. The principal objective in the calculations that follow is to show that the bacteria are capable of consuming a significant portion of the hydrogen which is released at the cathode. This would strengthen the inference drawn previously that cathodic polarization is lessened upon removal, not necessarily total removal, of molecular hydrogen by bacteria and diffusion plus circulation. Of course, other surface conditions, e.g., involving factors such as adsorbed gases affecting possible cathodic catalytic action, would remain to cause departure from the reversible hydrogen potential during the kinetic action which accompanies the delivery of power to the load.

Hydrogen Evolution - From the hydrogen reaction, $2H_2O + 2e^- = H_2 + 2OH^-$, it is apparent that one mole of H_2 is produced by 2F electrons. In order to express gas evolution in the more convenient form of a rate, such as cubic centimeters per hour, the 2F factor is converted to ampere-hours. This yields $2F/3600 = 53.3$ ampere-hours. Since one mole of H_2 equals 2.016 g and since there are 0.09 g/liter, then

$$\frac{2.016}{53.3} \frac{g}{amp\cdot hr} \times \frac{1}{0.09} \frac{liters}{g} = 0.42 \text{ liter/amp}\cdot\text{hr} = 420 \text{ cc/amp}\cdot\text{hr.}$$

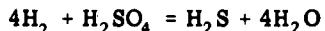
Cathodic hydrogen evolution estimated from the Armour work (8) is 410 cc/amp·hr at 1.0 amp/ft², and 450 cc/amp·hr at 0.5 amp/ft² current densities. These results merely show good agreement between stoichiometry and observed operation of the hydrogen reaction proposed for the biocell. It follows that cathodic hydrogen evolution in the biocell B of Fig. 2 would be

$$4.2 \text{ cc/hr at } 0.01 \text{ amp (0.1 amp/ft}^2\text{)}$$

$$21.0 \text{ cc/hr at } 0.05 \text{ amp (0.5 amp/ft}^2\text{)}$$

$$42.0 \text{ cc/hr at } 0.10 \text{ amp (1.0 amp/ft}^2\text{).}$$

The 4.2 cc/hr figure will be used as a check on bacterial hydrogen uptake, since Fig. 2 shows that the polarization increases more rapidly beyond the 0.1 amp/ft² current density, indicating that the hydrogen uptake of the quantity of bacteria present is reaching saturation.

Hydrogen Consumption - A stoichiometric expression

has been shown to be a fairly good representation of the uptake of hydrogen with respect to the efflux of hydrogen sulfide for some species of *Desulfovibrio*. The ratio $\text{H}_2/\text{H}_2\text{S}$ has been checked as close as 4.1 for a pure culture and found to range up to 8.9 among those mixed cultures studied (10).

The hydrogen uptake capability for various cultures and species of marine *Desulfovibrio* have been determined with the following results:

$$8.5 < Q_{\text{H}_2} \text{ cc/hr} < 34.7 \text{ for pure strains (10)}$$

$$13.8 < Q_{\text{H}_2} \text{ cc/hr} < 18.5 \text{ for mixed cultures (10)}$$

$$2.5 < Q_{\text{H}_2} \text{ cc/hr} < 36.0 \text{ for } D. \text{ aestuarii (7),}$$

where Q_{H_2} is the hydrogen absorption coefficient expressed as cc of hydrogen absorbed per milligram dry weight of a suspension of resting cells (7). The Q_{H_2} for *D. desulfuricans* has been reported as high as 340 (7).

Two estimates will be made of the quantity of bacteria present for the biocell B of Fig. 2. Based on visual inspection of this biocell, a 0.1-mm thickness is estimated over the 0.1- ft^2 surface for the matte-like substance containing the bacterial colony and associated products such as sulfides. The total volume of the film is calculated to be approximately 1 cc. Estimating that one-thousandth of this volume is occupied by bacteria whose specific gravity is about that of water, we arrive at a figure of 1 milligram for the weight of the bacteria. From the previously stated Q_{H_2} values ranging from 2.5 to 36.0, it is observed that the bacteria have at least the capability to take up approximately all the hydrogen evolved, with the effects of diffusion and circulation included in this estimate.

The second estimate of the amount of bacteria present draws on the determination made elsewhere (11) of the density of bacteria on a cell carrying a current density of about 0.1 amp/ ft^2 . The count was 2.87×10^{13} *Desulfovibrio* per gram of FeS matrix which was scraped off the biocell. The nickel sulfide was scraped from the active side of a biocell whose characteristics are described later in Fig. 6; and for practical purposes it is noted that this biocell is much the same as biocell B in Fig. 2. The NiS measured 1.51 grams. Assuming a lesser volume and amount of particulate matter per unit weight, and basing this change on the ratios of specific gravities for FeS and NiS, we adjust the above bacterial density to $4.8/5.5 \times 2.87 \times 10^{13} = 2.5 \times 10^{13}$ bacteria/gram of NiS. For 1.51 g of NiS, there would be $2.5 \times 10^{13} \times 1.51 = 3.78 \times 10^{13}$ bacteria. Assuming also a bacterium dimension of 0.5 micron diameter \times 2 microns length results in a total bacteria volume of approximately 0.015×10^{-3} cc. Requiring a specific gravity for the bacteria of 1.025, i.e., equal to that of seawater, yields a total weight of 15.4 mg.

Since the Q_{H_2} value has been previously shown to vary between 2.5 and 36.0, the bacterial weight of 15.4 mg is capable of a hydrogen uptake ranging from 38.5 to 550 cc/hr, which would exceed the 4.2-cc/hr requirement for the biocell B of Fig. 2 at 0.1 amp/ ft^2 current density. This hydrogen uptake estimate would bracket the requirement even though the cell count had been too high by a factor of 100.

Therefore the two estimates have yielded the following values.

Estimate of Desulfovibrio quantity	H ₂ uptake (cc/hr)	Requirement (cc/hr at 0.1 amp/ft ²)
1 mg	2.5 - 36.0	4.2
2.5 × 10 ¹³ per g NiS	38.5 - 550	4.2

Reproducibility of Performance

The following data are presented to give an indication of how storage affects the performance of these biocells. The presentation is limited to off and on use of a given cell over a six-month period and to the comparison of two cells prepared at the same time, i.e., having cathodes cultured simultaneously in the same growth media.

Figure 4 shows a general degradation on the basis of tests repeated at the end of the four- and six-month time lapse following the initial test. The significant portion of the curves extends from the 0.5 to 2.0 amp/ft² current densities. All biocell curves lie above that of the control, also shown. In the intervals between tests the biocell cathode was stored in a sealed container at room temperature in artificial seawater, Sea-Rite, with only residual amounts of nutrient present.

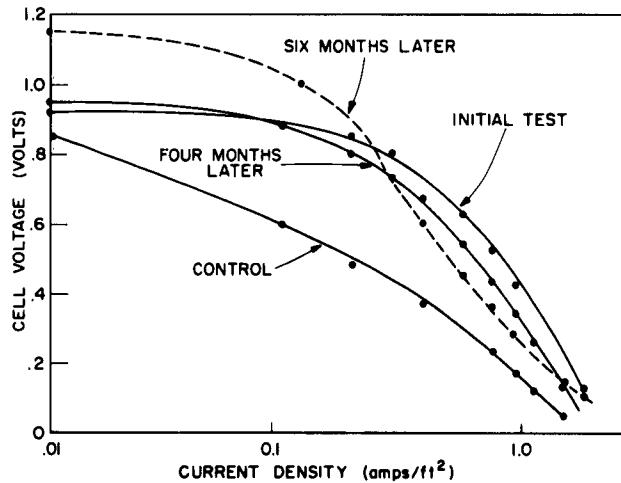


Fig. 4 - Repeatability of biocell performance characteristics

To exemplify performance consistency among cells, Fig. 5 presents two characteristics for two biocells whose cathodes were prepared, i.e., cultured with a bacterial coating, at the same time in a common culturing medium. The similarity is obvious, as well as striking. Experiments with these cells have disclosed consistent repetition of performance for cells of like construction, the greatest disparity among them occurring at current densities below 0.1 or 0.2 amp/ft². By far the greatest reason for departure among biocell characteristics is due to the properties of the basic magnesium seawater cell, notably the condition of the cathode surface or substrate on which the bacterial colony rests, and particularly at low currents, to the cleanliness of the magnesium. As an example of this, Fig. 6

shows the difference between cells with plain mild steel and nickel-plated cathodes. In this case, the bacterial colonies were cultured at different times and locations, thus adding some further uncertainty to the difference in results. The differences in ordinate intercepts is due mainly to the difference in condition of the magnesium anodes. Were the anodes alike, the lower curve would fall more nearly along the dotted line at low current densities.

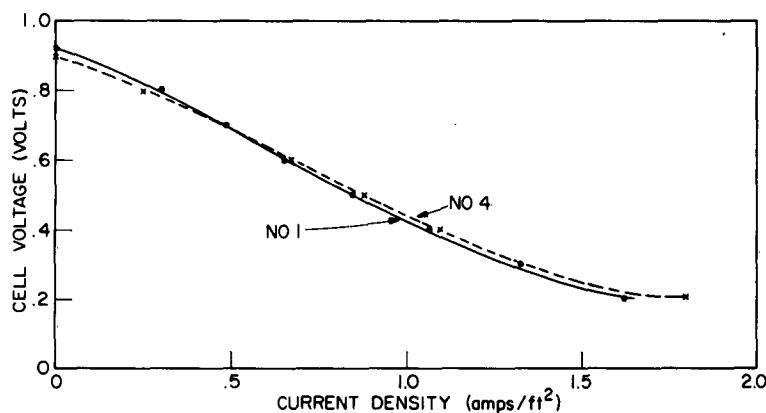


Fig. 5 - Comparison of a linear scale of two biocells with cathodes cultured from the same lot

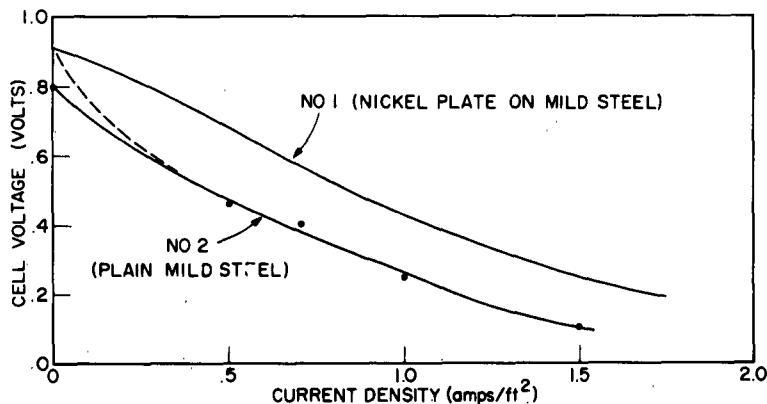


Fig. 6 - Effect of the cathode surface on biocell performance

Aging as a result of long-term operation imposes the next greatest change in the voltage-current characteristic. An attempt will be made to deal with the factors contributing to this change and will be discussed in a later section.

EFFECTS OF TEMPERATURE

By raising the temperature it is possible to determine further the effects of the bacteria. Ultimately, of course, the bacteria will be killed, permitting the observation of performance of a cell identical to the biocell but without the benefit of bacterial action.

Figure 7 shows how a biocell behaves as the temperature is raised. A marked reduction is observed in the step from 20°C (room temperature) to 25°C. This may be due to the fact that the bacteria were cultured at room temperature and consist of strains with maximum viability there. Optimum growth for *D. aestuarii* has been reported to be in the 25° to 30°C range (12) with little tolerance to temperature above 45°C. The pH condition of approximately 9.0 existing during these tests may have offset any possible benefit of higher temperatures in the 25° to 30°C range, since the seawater species of *Desulfovibrio*, i.e., *aestuarii*, is reported to have a pH of 8.5 as a growth limit with an optimum between 6.0 and 8.0 (12). Tests on the type of *Desulfovibrio* used here revealed optimum performance at about pH = 6.0 to 6.5 in biocell application (11).

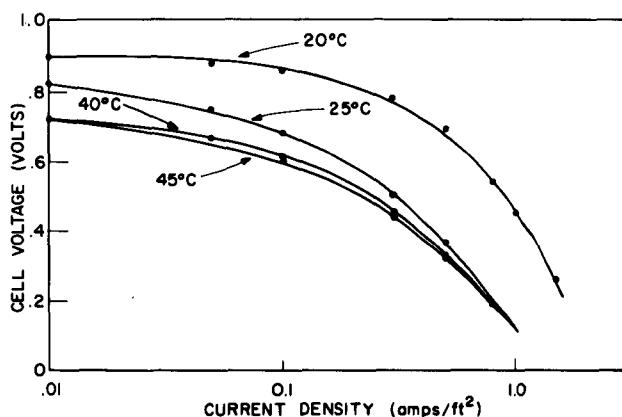


Fig. 7 - Effect of temperature on biocell performance

The further reduction in performance is noted in Fig. 7 by the curves at 40° and 45°C. Two effects are suspected in connection with the lessening increments of performance degradation with temperature increase. For one, the increase in cell temperature boosts the reaction rates and hence the electrochemical conversion. Figure 8 shows how further temperature increases up to 85°C result in increased performance. For another, the initial killing, or at least growth retardation, effects the bulk of the culture which is being subjected to temperature increase. One might well conclude that the culture, which is probably a mixture of strains, has a survival distribution humped at room temperature, or at the original culturing temperature and tapering off at higher temperatures. Any temperature increase beyond 30°C, say, will therefore exhibit less incremental effect. That is, the depolarizing action will persist in smaller successive increments.

After the biocell has been subjected to heating up to 85°C, it was allowed to cool to room temperature. The result is shown in Fig. 9. This figure shows clearly that once the bacteria have been killed the performance not only is degraded, but also it assumes a level resembling that of the control shown by the dashed curve. The significance of this in relation to the nature of the cathode surface will be brought out in the subsequent section.

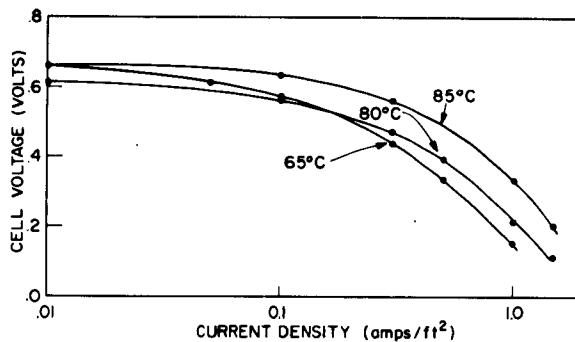


Fig. 8 - Further effect of temperature showing increased electrochemical activity

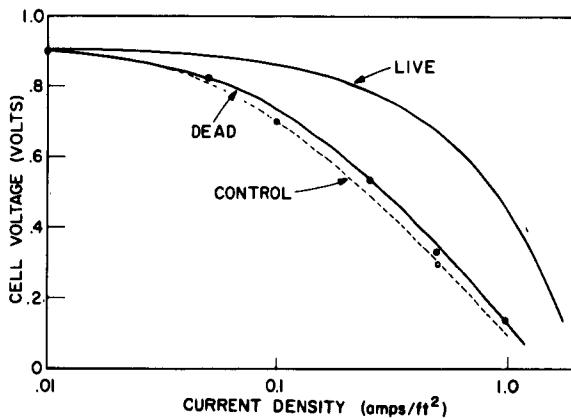


Fig. 9 - Comparison of the cell with live cultures and the cell with dead cultures at 20°C

Present in the characteristics is an inherent error which is difficult to deal with quantitatively, but which must be discussed. It involves an additional factor of aging which actually exaggerates the disparities among the curves of Fig. 7 and is one of the chief reasons for the differences between the sets of curves A and B in Figs. 2 and 3. Upon prolonged operation additional polarization is noted at both the cathode and anode, particularly in the latter. Carbonates are suspected of causing this additional performance deterioration. Tests in duration of a week or more have produced a calcareous scale which has been analyzed as consisting principally of magnesium carbonate. This scale was formed on both the anode and cathode. Such scale, known as Cox-coating, has been deliberately formed to inhibit corrosion on ships. It has also been reported in connection with the operation of the plain magnesium seawater cell (13, 14), along with a method for its removal by reversing the current in the cell.

No attempt was made to separate this effect from that of the bacterial depolarization in transcribing the characteristics of Figs. 7 and 8. However, prior to measuring the cell with the dead culture shown in Fig. 9, the cell was disassembled, and the anode cleaned,

reassembled, and flushed vigorously. Although the cathodic deposit of carbonate undoubtedly remains, its position atop the culture-sulfide matrix apparently renders this deposit more porous and thus less of a barrier to create polarization. This seems true at least in these laboratory tests. Actual seawater tests seem to be accompanied by a more formidable coating described later. This cell refreshening has unfailingly restored the cells to a condition where reproducibility of the current-voltage curve could be achieved. Thus the general diminution of performance with aging appears to be due chiefly to fouling at the anode and less to changing conditions at the cathode surface. For even longer term operation in seawater, additional marine fouling further deters performance as discussed later.

RELIABILITY OF THE CONTROL

It has been argued that the control cell is not in reality the exact bacterialess counterpart of the biocell. That is, a cell constructed identically to a biocell, but not having undergone the bacterial culturing process on its cathode, performs differently than the biocell from which only the effects of live bacteria have been removed. This argument is based on the premise that the bacterial culturing conditions the cathode surface in such a way that as a cathode, even devoid of live bacterial habitation, it may behave differently than the supposed identical control. However, the experiments reported here show that the control does indeed serve as it is intended, i.e., to perform as would a biocell with only its bacterial action suspended. To a great extent this is to be expected, since the cathode functions as an inert electrode in the first place.

Figure 10 shows several characteristics for cells designed for use as controls and for use as biocells. The similarity of all curves indicates that the killing of the culture in the biocell reverts it to the status of a control; that is, an electrode covered with a matrix of killed culture behaves nearly identically to that of the same electrode with the matrix scraped off.

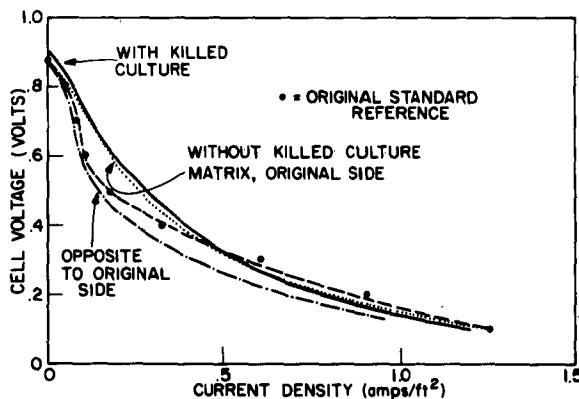
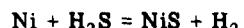


Fig. 10 - Comparison of the cell with and without a culture matrix

When a bacterial culture is grown on the electrode to be employed as a cathode, it covers this electrode with a crust. The thickness of this encrustation does not seem to determine the quality of the cathode. That is, a thin, more or less transparent, coating of bacteria has been as effective in depolarizing the hydrogen overvoltage as a thick, matte-like deposit. The latter has generally resulted from longer culturing periods.

The composition of the crust is undoubtedly a mixture, or matrix, of bacterial excrement, deposits of visible and dead cells, and products formed from the reaction of bacterial by-products with the electrode. For example, hydrogen sulfide is known to be a product in the genus *Desulfovibrio* metabolism. The oxidizing relations



would follow depending on whether the cathode surface was mild steel or nickel. The black sulfide is characteristic of the cultured cathodes. The formation of the sulfide layer, while altering the cathode surface, and involving electron transfer in the reactions in Eq. (4), does not alter cell performance to an appreciable extent as is apparent from Fig. 10. Furthermore this process is limiting in that the sulfide layer in time tends to block the H_2S from reaching the Fe or Ni. The sulfide is a good conductor, and hence the cathode retains its essential characteristics as an electrode.

SCHEMATIC INTERPRETATION OF BIOCELL ACTION

Experiments on the biocells fail to disclose other than a cathodic depolarizing action due to the bacteria. This conclusion is drawn from the observations, however insufficient, that the biocell terminal voltage for finite cell currents is (a) above that of the control or biocell with its culture killed, and (b) equal to the control at zero current. This latter equality is, of course, inferred from the average of measurements at very low currents, but is nevertheless a consistent observation. On these premises it is possible to depict the operation of the biocell as that of an ordinary magnesium seawater cell upon which the depolarizing effect of the bacteria is superimposed. In alternative terms, the presence of bacteria lower the internal resistance of the plain magnesium seawater cell.

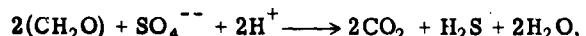
The reactions in Eq. (3) described previously for the magnesium seawater cell apply also to the biocell action. In addition, an expression must be furnished to account for the action of the bacteria as it relates to their role in the biocell. One such relation may be described by (4,15)



This equation is balanced for convenience but does not necessarily represent the stoichiometry involved in the bacterial metabolism. For a pure *Desulfovibrio* culture reported by Sisler (10), this stoichiometry has been confirmed by determining the $\text{H}_2/\text{H}_2\text{S}$ ratio. Mixed cultures show higher ratios, however. Hence Eq. (5) simply depicts the fact that the bacteria are known to take up molecular hydrogen, sulfate, and excrete hydrogen sulfide. Intermediate steps such as (15)



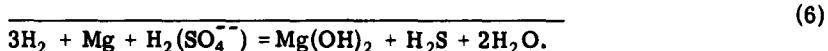
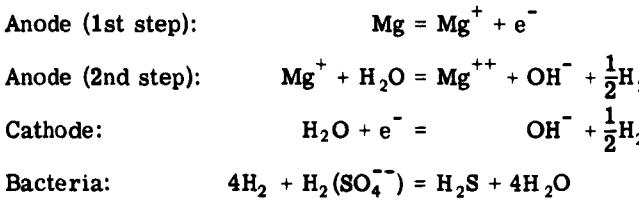
or more accurate representations of metabolism, as (16)



are allowed for, but these are considered as reactions taking place internal to the bacterium and assisted by enzymatic activation. The principal concern here involves merely the phenomenology of the biocell. Knowing the overall cell reactants and the quantities involved in bacterial ingestion and effluent, we may view the bacterial colony externally, and its connection with biocell performance can be made just sufficient to explain performance

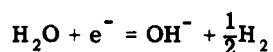
adequately. Although it is not always advisable to resort to a black-box concept for the bacteria function, a consideration of only the selective chemical inputs and outputs relevant to the cell's electrochemical performance seems sufficient for the purposes of deriving the mechanistic model proposed here. On this basis, Eq. (5) is chosen to be added to Eq. (3) to form the constitutive set of biocell equations.

The complete set is summarized as follows:

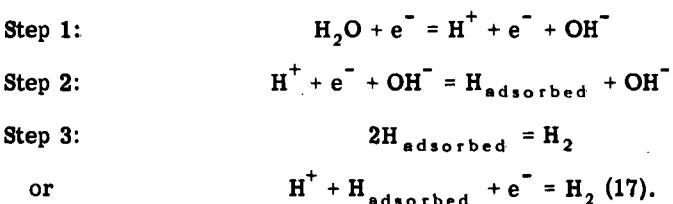


As in Eq. (3) equal quantities of hydrogen are evolved at the cathode and anode, as confirmed by the Armour studies (8). However, the stoichiometry of the net reaction equation is not established. For example, at higher current densities, saturation of hydrogen uptake by the bacteria occurs, preceded, of course, by a diminishing percentage uptake relative to the total hydrogen evolved at the cathode. Also at higher current densities, hydrogen evolution at the cathode exceeds that at the anode. Therefore Eq. (6) must be considered only qualitatively.

The cathodic reaction



gives rise to the hydrogen overvoltage, which is being lessened by the catalytic properties of the cathode surface and bacterial action to an extent dependent on bacterial viability and the hydrogen uptake capacity of the bacterial species relative to total hydrogen efflux. This reaction may be viewed from several steps:



Most likely the bacteria remove hydrogen predominantly in the molecular form, although ingestion in the atomic form has been speculated (7). But the required activation overvoltage component is probably due more to the adsorbed atomic hydrogen or effects attributed to it. Furthermore, the bacteria are mostly situated at large distances, in terms of intermolecular dimensions, from the adsorbed hydrogen. This statement assumes that the adsorbed hydrogen is located in the metallic and sulfide surfaces of the cathode. Hence the contribution made by the bacteria in reducing activation overvoltage would be due to the removal of H_2 , leading to a subsequent increase in the reaction rate of step 3. Since the understanding of hydrogen overvoltage is as yet unresolved (17), it is advisable here to avoid discussing it in detail. Rather, attention will be confined to relating bacterial action to factors in a manner at least consistent with experimental observations. This argument further relegates the role of the bacteria to an indirect one. It is only logical to postulate the action of the bacteria as merely coincidental to the function of the magnesium seawater

cell performance, fortuitously improving it, since the bacterial metabolism involving cell actions may take place at sites completely isolated from the cathode.

A schematic representation of the biocell is shown in Fig. 11. This model is merely an expansion of that for the common magnesium seawater cell shown in Fig. 1.

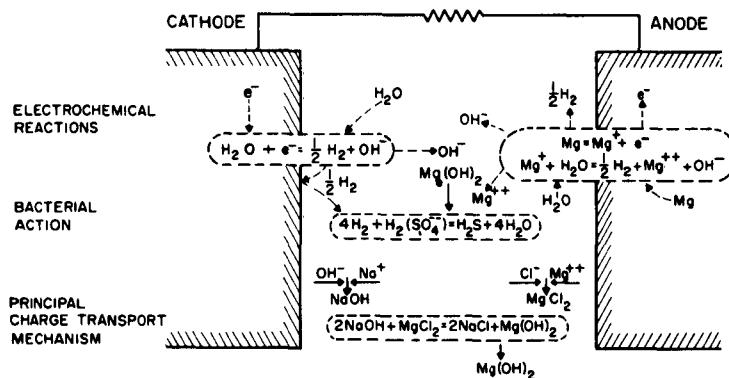


Fig. 11 - Schematic representation of the biocell

SOME RESULTS OF LONG-TERM TESTS

Figure 2 has already illustrated how biocell performance can fall off while in operation for periods just over one week in duration. These results utilized actual seawater whose pH had dropped off to about 6.5 from an initial value of over 8.0 while in storage and thus represented operation under near optimum pH conditions (11).

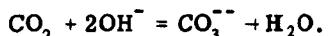
The difficulties with the logistics of supplying seawater, real or artificial, over long periods, even to small cells, led to a life test in an ocean site. Simple two-plate cells were employed, two being biocells and a third serving as the plain magnesium cell control. The electrodes were 0.25 ft² in area. The load was adjusted initially to hold the cathode potential constant at V(SCE) = 0.83 volt. The resultant cell voltage was 0.73 volt; the current value, 0.021 amp, or 0.084 amp/ft² in density; and the power density, 0.061 watt/ft². The object here was not to achieve maximum power but to keep the cathode potential most favorable for maintenance of the bacterial colony, 0.8 volt being approximately the value at which the cathode was kept during culturing. Interestingly, the current density is close to the 0.1 value upon which much of the previous discussion is based, particularly in connection with the hydrogen uptake capacity of the bacteria.

This output level was not maintained for long. In a matter of hours the output voltage dropped to 0.5 volt, or about the same voltage as the control. Roughly at weekly intervals, the biocell voltage would rise to as high as 0.6 or 0.7 volt. At the end of one month, the biocell stabilized at approximately 0.45 volt. The relatively stable control voltage had dropped from 0.5 to 0.4 volt. During subsequent operation, the output voltages of the biocell continued to fall off. Approximately three months later, a total of five months from the start of the life tests, the terminal voltage of both the biocell and its control had dropped to approximately zero.

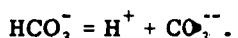
The gradual falloff in performance is the significant result in these tests. Calcareous deposits have been known to reduce performance (13). However, it was speculated early

in the biocell studies that the bacterial matrix would deter any such formation, or perhaps keep it porous to charge carrier flow. The performance results show that a polarization buildup occurs slowly as operation in seawater persists. This may be due to carbonates either retarding the reaction at both the anode and cathode or impeding ion flow or both. White deposits bearing at least a visible similarity to carbonates are observed at both the anode and cathode active surfaces, i.e., facing the electrolyte in the interelectrode space.

Upon spectrochemical analysis, the white deposits were revealed to be strong in magnesium and calcium content. All other elements detected in the analysis showed no preferential location, whereas Mg and Ca were present most abundantly in the active vicinity of the electrodes. The simultaneous presence of OH^- at the electrode sites, coupled with the high solubility of CO_2 in seawater make the following reactions probable:



The first expression can be carried further to



The equilibrium constant is low for this reaction, about 5×10^{-11} , but this entire carbonate formation phenomenon is observed to be a slow process. A third possibility exists in the reaction



the HCO_3^- again going to H^+ and CO_3^{2-} . Thus all reactants are present for formation of magnesium and calcium carbonates.

Another distinct layer was observed covering the entire cell, supporting structure as well as the surfaces adjacent to the interelectrode space. It was densest on the inner cathode surface, but was attached spottily to the magnesium. This layer bears a resemblance to that produced by film-forming organisms (9) known to exist over a wide range of geographical latitudes and oceanic depths. Wet or dry it behaves as an insulator, has relatively low porosity, and thus could well contribute to the general degradation of the biocell performance over the course of the five-month life test. The spectrochemical analysis of this layer revealed the presence also of calcium and magnesium, and silicon as well. Therefore it is likely that this is a calcareous film resulting from natural processes in the sea. The bacterial colony did not deter the formation of this film.

Although $\text{Mg}(\text{OH})_2$ is copiously present, it has a strong tendency to remain in colloidal suspension, is easily carried away with the continuously flowing seawater electrolyte, and appears relatively nonadherent to the electrode. A certain amount collects on the electrodes, of course, but does not appear to cake up or coat as does a carbonate.

SUMMARY

These experiments have defined certain characteristics of the biocell relevant to its use as an electric power source. The results are, of course, restricted to the specific type of cell described in the text. However, the capabilities and limitations in performance presented here typify those one may expect in general from this class of biocell.

The gain in performance of the biocell over that of the plain magnesium seawater cell, both well designed to function as an electric source, is best considered as an increase in power due to increased terminal voltage. Both operate over substantially the same current

range for like configuration. It is estimated that the action of bacteria superimposed on a well-designed magnesium seawater cell, whose cathode has been plated, say with nickel, would increase the terminal voltage to 0.8 volt from a value of 0.5 volt at 0.5 amp/ft² current density, thereby increasing the power output density from 0.25 to 0.40 watt/ft².

Energy factors would change according to the lessening of dissipation with reduced internal resistance. Table 2 compares these factors. From these figures one may conclude that were it not for the aging effects observed in long-term tests, the biocell would offer an advantage over the plain Mg cell for low-power, long-term applications. Figures on the Leclanche and lead acid cells are added for reference. However it is emphasized here that they are not intended to be used as comparisons with the seawater cells. It is uncertain whether questions of wet or dry weight of the seawater cell, and its state of development and fabrication are properly included in this estimate.

Table 2
Comparison of Energy Factors

Type of Cell	Voltage (volts)	Current Density (amp/ft ²)	Energy Density (watt-hr per lb)	Weight (lb per watt-year)	Cost per Watt-Year (\$)
Mg seawater Cell*	0.5	1.0†	146	60	60
Biocell†	0.8	1.0	240	36.5	36.5§
Leclanche	1.5		23	390	200
Lead Acid	2.0		20	430	250

*Assumed to be well designed. Data for this cell derived from Ref. 14.

†Idealized estimates based on improvement of basic magnesium cell upon which bacterial action is superimposed.

‡Coincidentally near maximum power point for cells of Fig. 3.

§Plus culturing, care, and feeding of bacteria.

The 2 to 1 gain in power of the biocell over the magnesium seawater cell for current densities below 1 amp/ft² is small compared to power densities reported by Goldenberg (18) for magnesium seawater cells of special design. He claims a current density of 25 amp/ft² at 0.5 volt, a value which far exceeds the bacterial capabilities observed in these experiments. The energy factors would, of course, parallel those for the magnesium seawater cell.

Is it meaningful to make direct comparisons between biocells and plain magnesium seawater cells? The answer is affirmative only if the designs of both are reducible to an equivalent basis for comparison. It is assumed that both are well designed for their purposes. Factors such as close spacing, dynamics of electrolyte flow, and pH gradients, which are present in optimally designed magnesium seawater cells, may be incompatible with conditions necessary for proper bacterial function.

Finally, although the bacteria are indigenous to the sea, they are not compatible to the sea's environment in the sense that they continue to function as depolarizers in the type of electric power source described here. It appears that film-forming organisms shut down the cell's entire electrochemical action and that this blocking action commences as early as a few hours after start up. Also a previous conclusion was that the bacteria give no evidence of converting the sea's vast resources to electricity by direct action on their part.

ACKNOWLEDGMENTS

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<p>UNCLASSIFIED</p> <p>Naval Research Laboratory. Report 5998. EXPERIMENTS WITH A MAGNESIUM SEAWATER CELL INCORPORATING A BACTERIAL COLONIZED CATHODE, by B. J. Wilson. 22 pp. and figs., October 4, 1963.</p> <p>A magnesium seawater cell, with its cathode covered by a colony of special bacteria, yields a higher voltage at a given current density than when bacteria are not present. This has been observed for current densities below one ampere per square foot. Volt-ampere characteristics were measured to determine how much the voltage performance improved, and if possible, why. On the basis of these tests, it is possible to conclude that the bacteria act to lessen cathodic polarization, and therefore the equivalent internal UNCLASSIFIED (over)</p>	<p>UNCLASSIFIED</p> <p>Naval Research Laboratory. Report 5998. EXPERIMENTS WITH A MAGNESIUM SEAWATER CELL INCORPORATING A BACTERIAL COLONIZED CATHODE, by B. J. Wilson. 22 pp. and figs., October 4, 1963.</p> <p>A magnesium seawater cell, with its cathode cov-ered by a colony of special bacteria, yields a higher voltage at a given current density than when bacteria are not present. This has been observed for current densities below one ampere per square foot. Volt-ampere characteristics were measured to determine how much the voltage performance improved, and if possible, why. On the basis of these tests, it is possible to conclude that the bacteria act to lessen cathodic po-larization, and therefore the equivalent internal UNCLASSIFIED (over)</p>	<p>UNCLASSIFIED</p> <p>Naval Research Laboratory. Report 5998. EXPERIMENTS WITH A MAGNESIUM SEAWATER CELL INCORPORATING A BACTERIAL COLONIZED CATHODE, by B. J. Wilson. 22 pp. and figs., October 4, 1963.</p> <p>A magnesium seawater cell, with its cathode cov-ered by a colony of special bacteria, yields a higher voltage at a given current density than when bacteria are not present. This has been observed for current densities below one ampere per square foot. Volt-ampere characteristics were measured to determine how much the voltage performance improved, and if possible, why. On the basis of these tests, it is possible to conclude that the bacteria act to lessen cathodic po-larization, and therefore the equivalent internal UNCLASSIFIED (over)</p>
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